

Nigella sativa supplementation attenuates exercise-induced bronchoconstriction in the maturing rat: a histometric and histologic study

Ehsan Arabzadeh¹ · Shadmehr Mirdar² · Hiwa Moradiani¹

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Abstract Recent evidence suggests that *Nigella sativa* (NS) has an antiasthmatic (bronchodilatory) effect in the airways of asthmatic patients. Our objective was investigation of the hypothesis that NS supplementation reduces exercise-induced bronchoconstriction (EIB) in the maturing rat after high-intensity interval training (HIIT). Forty male Wistar rats (aged 3 weeks) participated in this study. The animals after 2-week adaptation were divided into three groups: Base, control HIIT, and HIIT (HIIT and NS group). HIIT group was performed on a treadmill (20 to 70 m/min, 30 min/day, 6 days/week). After 6 weeks HIIT, NS seed ethanol extract was fed by gavages to NS group 500 mg/kg for 3 weeks. Finally, animals were euthanized and their lung removed, weighted, and fixed in 10 % formalin. By routine and standard paraffin embedding, lung samples were sectioned in 5 µm and stained with H&E and studied histologically and histometrically. Massive inflammation and characteristics of lung remodeling including goblet cell hyperplasia, lymphocyte infiltration, and severe emphysema were observed in the lungs of HIIT-exposed rats. Administration of NS in the HIIT-exposed rats suppressed the inflammatory cell infiltration, goblet cell hyperplasia, and thickness tunica intima, tunica media, and tunica adventitia bronchi significantly. Administration of NS reduced the level of airway inflammation bronchi-induced asthma. The effect of NS on EIB is probably mediated by the reduce thicknesses intima, media,

and adventitia and the number of goblet cells of the bronchi. Our findings suggest that NS might have useful implications in the treatment and future research into EIB.

Keywords *Nigella sativa* · Exercise-induced bronchoconstriction · Maturing · High-intensity interval training

Introduction

Airway epithelial cells play an important role as a physical barrier against environmental toxins and damages and modulate inflammation and immune response. Some of the studies suggested that exercise can lead to injury in the airway epithelium (Kippelen et al. 2012). Exercise-induced bronchoconstriction (EIB) is a transient narrowing of the lower airways after intense exercise which increased in elite athletes markedly (Elkins and Brannan 2012). EIB is a result of evaporative water loss in conditioning the inspired air. The water loss causes cooling and dehydration of the airway surface. The acute effect of dehydration causes to release mediators, such as leukotriene, histamine, and prostaglandins that can stimulate smooth muscle and alteration in vascular permeability and contractility (Anderson and Kippelen 2008).

Whereas the potential triggering factors for EIB are well recognized, the basal pathogenesis is less unknown (Anderson and Kippelen 2005). It usually occurs after at least several minutes of intense “aerobic” exercise, which demands of normal nasal breathing be supplemented by mouth breathing. The consequent inspiration of air that has not been warmed and humidified by the nasal passages seems to generate elevated blood flow to the linings of the bronchial tree, resulting in edema. Structure of these small airways then follows, worsening the degree of block to airflow. There is increasing

✉ Ehsan Arabzadeh
eh.arabzadeh@gmail.com

¹ Department of Cellular and Molecular Exercise Physiology, Faculty of Physical Education and Sport Science, Urmia University, Urmia, Iran

² Department of Exercise Physiology, Faculty of Physical Education and Sport Science, University of Mazandaran, Babolsar, Iran

evidence that the smooth muscle of lines in the airways becomes gradually more sensitive to changes that occur as a result of damage to the airways from dehydration. The chemical mediators that provoke the muscle spasm appear to be secreted from mast cells (Anderson and Kippelen 2005). Exercise does not cause asthma but is frequently an asthma trigger (Khan 2012). EIB can happen in people with or without chronic asthma, although it is highly prevalent in people with asthma (Elkins and Brannan 2012).

In the past few years, there are many antiasthmatic medications that have caused effective anti-inflammatory medicines being utilized for the treatment of mild to moderate persistent asthma. However, their use also raises concerns over side effects and compliance issues (Anderson and Kippelen 2012). Therefore, new medicines are needed, which should be safe, specific, and powerful. Currently, many medicinal herbs are available for the therapeutic action of various illnesses (Knöpfli et al. 2007). Among natural products, the seeds and oil of *Nigella sativa* (NS) have attracted the interest of medical scientists (Bougault and Boulet 2012). NS belongs to the Ranunculaceae family, which is an annual herbaceous plant with black seeds. The black seeds of NS have been approved as a flavor and food preservative in ancient cultures. The absolute studies have reported that black seed oil and its different components, such as thymoquinone, apparent anti-inflammatory, antitumor, immune stimulatory, and healing traits (Ahmad et al. 2013). In addition, oral administration of black seed oil can diminish the disease scores in patients with allergic rhinitis, bronchial asthma, and atopic eczema (Fitch 2012).

In previous studies reported, NS has a preventive effect on tracheal responsiveness and lung inflammation, modifies leukotriene synthesis, and inhibits histamine release (Bougault et al. 2011). Previously, it was suggested that pure NS supplementation may inhibit the allergen-induced airway inflammation (Boulet 2012; Chimenti et al. 2007) and we hypothesized that it might be able to attenuate the EIB in the maturing rat. Therefore, we used high-intensity interval training (HIIT) to induce EIB in rats in order to investigate the effects of NS supplementation on lung remodeling in acute EIB conditions.

Materials and methods

Animals

Forty newborn male Wistar rats (body weight, 68 ± 9 g; 3 weeks old) were maintained in stainless steel wire cages at constant temperature (23 ± 2 °C), humidity (45–55 %), and a 12-h light/dark schedule. Food and water were freely available (Bougault and Boulet 2012). When all animals were 3-week-old, they were familiarized with treadmill running for 2 weeks and then randomly assigned to a base group ($n=10$), control

HIIT ($n=10$) and HIIT group ($n=20$). After 6 weeks of HIIT, 10 rats of this group consumed NS for 3 weeks (NS group).

Exercise protocol

HIIT group underwent sprint training on the treadmill 5 to 6 days a week for 6 weeks. The running speed was gradually increased to 65–70 m/min with the number of sprint sets and resting periods being adjusted to enable all animals to run at the target speed. The exercise protocol is summarized in Table 1. The initial treadmill speed was 20–30 m/min in the first week and was increased gradually to 60–70 m/min for the final week. Each training session consisted of 10 sets of 1-min sprint running, and the sprint running sets were separated by 2-min recovery periods (Ogura et al. 2006).

Nigella sativa seed ethanol extract

In order to prepare NS seed extracts, drench method was used. Thus, 55 g of powdered NS was weighted using a scale with an accuracy of 0.001 mg/kg and was soaked in 70 % ethanol and 30 % distilled water solution for 72 h. During this period, the container was covered with a lid well-soaked into paraffin and was kept at room temperature (20 to 25 °C). The mixture was stirred with a glass rod every 6 h. After this period, the mixture was passed through a filter and the solvent was removed by rotary at a mild temperature (below 60 °C). After 6 weeks of HIIT, NS extract was fed by gavages to the NS group 500 mg/kg body weight for 3 weeks daily (Al-Ghamdi 2003).

Histologic and histometric experiments

The animal groups of base (before the start of exercise protocol), HIIT, and control HIIT (after the sixth week of HIIT) and NS groups (at the end of the third weeks of NS consumption) were anesthetized using the 3-unit injections of ketamine (50–30 mg per kg) and xylazine (5.3 mg per kg) solutions and were killed with a guillotine. Then, the lung tissue was removed using a surgical blade and weighted by a Sartorius BI 1500 balance with an accuracy of 0.001 mg/kg. Animals were euthanized, and their lung fixed in 10 % formalin. By routine and standard paraffin embedding, lung samples were sectioned in 5 μ m and stained with H&E and studied histologically and histometrically.

Statistical analysis

All data are presented as the mean $\pm$ SD. For assessment of differences among the groups, means used one way analysis of variance (ANOVA) with variance by Bonferroni post hoc. All statistical analyses were performed with a statistical

Table 1 Training program for HIIT group (speed: m/min)

Days in week	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Range of speed in week
Familiarization	^a	^a	10	15	20	25	^a	10–25
HIIT Week 1	20	25	25	^a	30	30	^a	20–30
Week 2	30	40	40	35	40	45	^a	30–45
Week 3	45	45	50	45	50	55	^a	45–55
Week 4	55	55	50	55	60	65	^a	50–65
Week 5	65	60	65	70	70	70	^a	60–70
Week 6	65	70	70	65	70	70	^a	65–70

^a Resting day

software (SPSS version 21.0). The significance level in all statistical analyses was set at $p \leq 0.05$.

Result

Lung weight

Body and lung wet weight increased with maturing rats. But only the HIIT group had lower weight changes. However, the relative weight was without increased in NS group as compared to the HIIT group (Table 2).

Histometric analysis

NS supplementation created significant decrease in histometric measurement ($p < 0.001$) (Table 3). NS group after 3-week NS supplementation in thickness epithelial bronchi, thickness tunica media (muscle) bronchi, thickness tunica adventitia bronchi, and number of goblet cells of the bronchi, respectively 41.48, 30.86, 56.50, and 39.28 % show decrease compared to HIIT group (Table 3).

Histologic analysis

Histological study results also showed that HIIT causes severe inflammation in the lung parenchyma and adventitia lung ducts. Three weeks of NS supplementation reduced the severity of injuries induced by HIIT and improved structure of the lung parenchyma (Fig. 1).

Table 2 Weight variable (mean $\pm$ SE)

Group variable	Base	Control HIIT	HIIT	NS
Body weight	64 $\pm$ 6.59	145.02 $\pm$ 3.96	123.1 $\pm$ 1.81	213 $\pm$ 17.51
Lung wet weight	0.69 $\pm$ 0.09	0.98 $\pm$ 0.05	0.69 $\pm$ 0.09	1.26 $\pm$ 0.06
Relative weight	0.01	0.006	0.005	0.05

Discussion

NS is widely known to have wide therapeutic applications in herbal medicine, and scientific advancement through technology has provided substantial evidence to support most of its medicinal claims. The present study has further demonstrated the antihistamine potential of this plant seeds which attenuates EIB. The data observed in Table 3 and Fig 1d demonstrate that NS supplementation after HIIT attenuated lung inflammatory responses in the maturing rat with EIB. Also, NS reduced epithelial cell hypertrophy and the number of goblet cells in bronchi duct, cellular infiltrate, and total protein concentration in the airway lumen (Fig. 1d).

The intensity and duration of exercise are key determinants of the airway response (Elkins and Brannan 2012). The increased air way responsiveness to prolong and high-intensity training seen in the present study is supported by previous studies indicating the effect of HIIT on respiratory system. Kippelen and Anderson (2012) show that severe exercise-induced hyperpnoea either by itself, or in combination with environmental stimuli, is a primary contributor to epithelial stress injury in competitive athletes. The influence of water and heat loss cause to the transient bronchoconstriction that occurs shortly after strenuous exercise is now widely accepted (Anderson and Daviskas 2000). Exercise requires increased ventilation, which results in respiratory water loss leading to airway drying and cooling. Evaporative water loss leading to an increase in the osmolarity of the airway surface liquid and consequent release of mediators is thought to be the major stimulus and mechanism for EIB (Anderson and Kippelen 2008; Elkins and Brannan 2012). The results of a previous study have shown that cold-weather athletes have airway hyperresponsiveness to pharmacologic agents as a result of epithelial injury (Anderson and Kippelen 2008).

Sometimes, athletes will take herbal supplements, or experiment with special diets to try to improve their performance, and they may even do this to improve their exercise asthma. The data supporting herbals or diets for exercise asthma or bronchoconstriction are very limited. There are preliminary data suggested antioxidants may have mild beneficial effect on EIB (Storms 2003; Neuman et al. 1999). Until further

Table 3 Mean ($\pm$ SE) variable of internal bronchi

Groups variable	Base	Control HIIT	HIIT	NS	<i>p</i> value
Thickness epithelial bronchi (μ m)	16.32 $\pm$ 1.86	23.4 $\pm$ 2.3	37.6 $\pm$ 2.51	23.6 $\pm$ 2.5	**
Thickness tunica media (muscle) bronchi (μ m)	23.98 $\pm$ 1.92	32 $\pm$ 2.74	46 $\pm$ 4.18	33.4 $\pm$ 2.59	**
Thickness tunica adventitia bronchi (μ m)	17 $\pm$ 2	27.6 $\pm$ 2.51	63 $\pm$ 3.46	28 $\pm$ 2.05	**
Number of goblet cells of the bronchi (per 100 μ m of epithelial cell)	9 $\pm$ 2	13 $\pm$ 2	22.4 $\pm$ 3.05	14 $\pm$ 1.15	**

** p <0.001 significantly different between groups

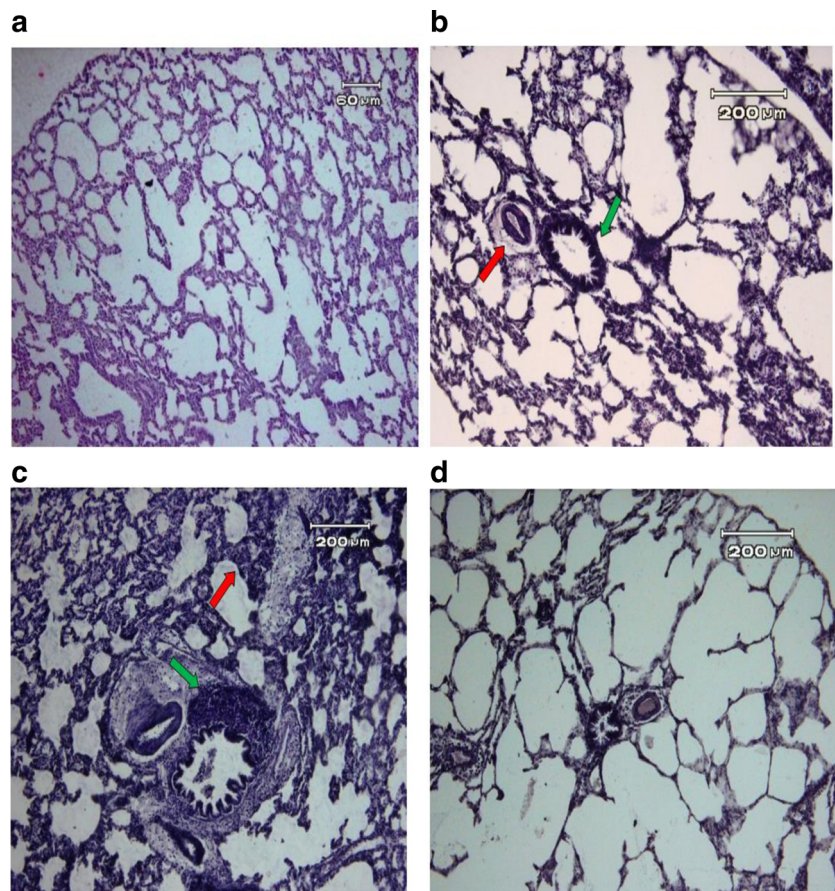
confirmatory studies are performed with long-term utilization of these therapies, it is premature to recommend supplements or special diets for athletes with exercise-induced bronchoconstriction. This is the first study to directly examine the role of NS supplementation on EIB. The results showed a relatively potent antiasthmatic effect of ethanol extract from NS. In fact, the extract caused significant decrease in all measured layers of the bronchi. The beneficial effects of NS oil on rats with hyperopia-induced lung injury were evaluated since oxygen-induced lung injury is believed to lead to the development of bronchi-pulmonary dysplasia in premature infant rats. NS oil reduced the severity of lung damage due to hypoxia significantly (Tayman et al. 2013). Gilani et al. (2001) showed that the bronchodilatory effect of the crude extract of

NS seeds was shown to be mediated possibly through calcium channel blockade (Gilani et al. 2001). It is suggested that nigellone but not TQ in NS may be useful in treatment of different respiratory diseases (Wienkotter et al. 2008). Nigellone, an active ingredient of NS, was found to effectively inhibit the histamine which is released from the mast cells (Gilani et al. 2004) and thus show the basis for its traditional use in asthma.

Conclusion

In summary, our study demonstrates that NS supplementation reduces EIB-related airway inflammation after HIIT and this

Fig. 1 Microscopic view of the lung tissue structure (H&E stain, $\times 200$ original magnification) following 6-week HIIT and 3-week NS supplementation. **a** Base: discipline in alveolar structure is visible. **b** Control HIIT: the histological structure of the lung parenchyma, air duct guiding, and respiratory tract is normal. *Green arrows* show bronchioles, and the *red arrows* show a pulmonary artery. **c** HIIT: severe inflammation of lung tissue, infiltration of inflammatory cells, and lymphocytes in the connective tissue surrounding the respiratory tract (*green arrows*) and interstitial blades (*red arrows*) are visible. Also, interstitial pneumonia and severe emphysema are visible. **d** NS: structure of the lung parenchyma is relatively normal, but the increase in alveolar surface compared to the other groups is visible



reduction may occur via antihistamine and bronchodilatory effects of the ethanol extract of NS seeds. These findings strongly suggest that ethanol extract of NS might be useful in the treatment of EIB in athletes that exercise extremely during a season of training.

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Conflict of interest The authors declare that they have no competing interests.

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